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The Detection of Rickettsia-Like Microorganisms Within the Ovaries of Female *Ixodes ricinus* Ticks

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Summary. An ultrastructural study of tick-borne fever (TBF)-infected *Ixodes ricinus* ticks revealed the presence of rickettsia-like microorganisms within the ooplasm and the mitochondria of developing oocytes.

These microorganisms are similar in appearance to the TBF agent and it is possible that although transovarial transmission of the TBF agent apparently does not occur, the rickettsiae are at least able to establish themselves in the ovaries of infected ticks.

Introduction

Cytoecetes phagocytophila, the causative agent of tick-borne fever (TBF), is transmitted by the sheep tick, *Ixodes ricinus.* Nymphae and adults which have fed, in their previous instar, on infected sheep transmit the disease to susceptible sheep on which they feed, whereas larvae hatching from eggs laid by females fed on infected sheep do not (Macleod and Gordon, 1933).

During an ultrastructural study of tick tissues, rickettsia-like microorganisms were discovered within the developing oocytes of TBF-infected *I. ricinus* females. In this paper a description of the microorganisms is given and their possible significance discussed.

Materials and Methods

The strain of *Ixodes ricinus* used in this investigation has been maintained in the laboratory for three years. It was originally obtained from the Central Veterinary Laboratory, Weybridge where it had been maintained for at least ten years.

Nymphae were infected with ovine TBF by feeding them on infected sheep and were allowed to moult to the adult instar. Ten adult females were fed on the ear of a rabbit and after five

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days were removed for dissection, which was carried out in Eagles minimal essential medium (MEM) supplemented with 20% ovine serum. Certain organs were homogenised for use in transmission studies, whilst the ovaries were removed and processed for electron microscopy.

Controls consisted of partially-engorged female *Ixodes ricinus* fed on an uninfected sheep.

Tissue was fixed for electron microscopy in cold (4° C) glutaraldehyde in cacodylate buffer (pH 7.2) and subsequently washed twice in cacodylate buffer (pH 7.2) before post-fixation in Millonig's (1961) osmium tetroxide solution. After washing in Millonig's buffer, tissue was dehydrated in a graded series of acetone and embedded in TAAB embedding medium (TAAB, Emmer Green, Reading). Glass knives were used to cut thin sections which were stained sequentially with uranyl acetate and lead citrate and examined on copper grids in an AEI 6B electron microscope, using an accelerating voltage of 80 kV.

Results

Rickettsia-like microorganisms were found in the cells of the ovarian epithelium, in the funicle cells of developing oocytes and, more commonly, in the oocytes themselves. The microorganisms were present in the ooplasm, either singly or in pairs, and in groups within the mitochondria (Fig. 1).

Microorganisms were not found in the ovaries of control ticks.

Microorganisms appeared coccoid, ranging in diameter from 0.2 μm to 1.0 μm and possessed two limiting membranes, the outer membrane often having a rippled appearance. The cytoplasm of individual microorganisms contained numerous ribosome-like granules interspersed with electron-lucent areas which occasionally displayed fine filamentous strands running through them (Fig. 3).

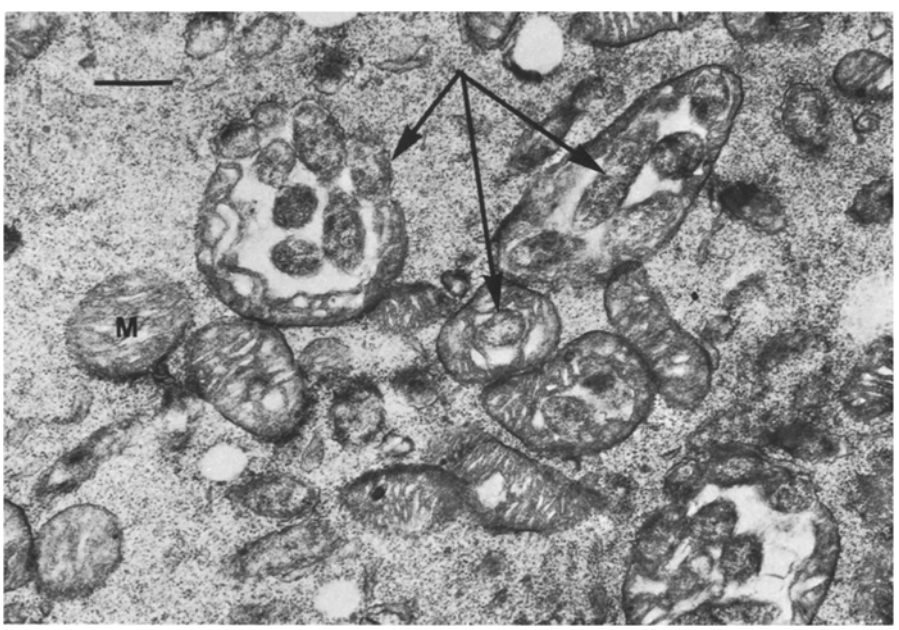

Fig. 1. Section through a developing oocyte in the ovary of a partially engorged adult female *Ixodes ricinus* tick. Mitochondrion *(M)*. *Arrows* indicate rickettsia-like microorganisms

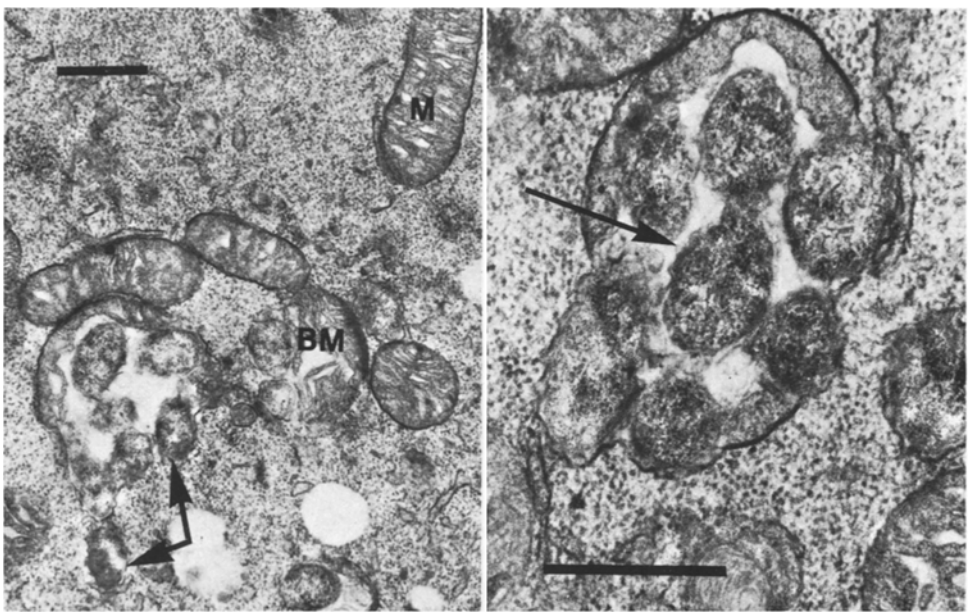

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Fig. 2. Mitochondria *(BM)* releasing microorganisms into the ooplasm display ruptured membranes and displaced cristae. Normal mitochondrion *(M)*. *Arrows* indicate microorganisms

Fig. 3. Rickettsia-like microorganisms are released into the ooplasm from a burst mitochondrion. The microorganisms exhibit rippled outer membranes *(arrow)* and numerous ribosome-like granules Bar in all cases represents 0.5 μm

Mitochondria which contained the microorganisms appeared swollen and their cristae were considerably displaced by them (Figs. 1 and 2). Some mitochondria appeared to have burst and to have released their microorganisms into the ooplasm (Fig. 3).

Discussion

The presence of rickettsia-like symbionts in the tissues of argasid and ixodid ticks has been reported by a number of authors (Balashov, 1972). Symbionts have been observed in the salivary glands, malphigian tubules and ovaries of several tick species (Roshdy, 1962, 1964; Reinhardt et al., 1972, and Burgdorfer et al., 1973).

In the current study microorganisms were found in the tissues of the ovaries of *I. ricinus* ticks known to be infected with *C. phagocytophila* but not in the tissues of the ovaries of control ticks from the same stock. These microorganisms exhibited a number of rickettsial characteristics. Their intracellular nature, electron lucent nucleoids, and double limiting membranes are all considered to be characteristic of the Rickettsiaceae. Furthermore they appeared identical to the TBF agent (Tuomi and von Bousdorff, 1966) and the closely related *C. ondiri,* the causal agent of bovine petechial fever (Krauss et al., 1972).

Although rickettsiae have previously been recorded from tick ovaries, their ability to exist within mitochondria has never been reported and their position in developing oocytes is said to be at the periphery, close to the shell (Roshdy, 1964; Burgdorfer et al., 1973). The microorganisms in the present study were more dispersed, but more importantly, they appeared to be derived from individuals multiplying in the mitochondria of the developing oocytes.

Salivary gland and gut homogenates derived from the ticks used in the present investigation produced typical TBF reactions when injected into susceptible sheep. It is possible, therefore that the microorganisms detected in the ovaries were TBF rickettsiae which had infected the developing oocytes during their dissemination through the body of the tick.

It is also feasible that oocytes so infected were not viable. Indeed, it is difficult to envisage the successful development of cells in which the mitochondria were affected to such an extent by rickettsial colonisation. This view is substantiated to some extent by the apparent expulsion of infected oocytes from the ovary of TBF-infected female *Ixodes ricinus* (Lewis, 1977) which would preclude the possibility of transovarian transmission of ovine TBF.

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